ISOLATION AND IDENTIFICATION OF 9-METHOXYCANTHIN-6-ONE COMPOUND FROM *Eurycoma longifolia* ROOTS

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ABSTRACT

9-Methoxycanthin-6-one compound has been isolated from Pasak Bumi (E. Longifolia) roots. The isolation process using maseration, colum chromatography vacuum, and recrystallization techniques. E. Longifolia root maserated with n-hexane and methanol respectively. The methanol extract fractionated using column chromatography vacuum with gradient elution (hexane-etil acetate) to obtain 9 fractions. Fraction 5 further purification using recrystallization technique to obtain yellow crystal, identified by LC-MS and NMR spectroscopy as 9-Methoxycanthin-6-one

Keyword: *E. Longifolia, isolation, spectroscopy, 9-Methoxycanthin-6-one.*

INTISARI

Telah dilakukan isolasi dan identifikasi senyawa 9-metoksisantin-6-one dari akar pasak bumi Eurycoma longifolia. Proses isolasi dilakukan menggunakan metoda maserasi dalam heksana dan metanol. Proses pemurnian dilakukan melalui teknik kromatografi kolom dan rekristalisasi. Hasil pemurnian ekstrak metanol melalui kolom kromatografi secara gradien menggunakan eluen heksana dan etil asetat menghasilkan 9 fraksi. Fraksi 5 selanjutnya dimurnikan menggunakan kromatografi kolom dan rekristalisasi menghasilkan kristal kuning. Senyawa tersebut diidentifikasi sebagai 9-metoksisantin-6-one, dengan menggunakan LC-MS dan FT-NMR.

Kata Kunci: *E. longifolia, isolasi, spektroskopi, 9-metoksisantin-6-one*

INTRODUCTION

The chemical constituents and the biological activities of ***Eurycomu longifolia*** Jack, a plant indigenous to Southeast Asia and much sought after as a herbal medicine, have been the subject of a number of investigations (***Chan, K.L., et al., 1992***) to generate considerable research interest because of its diverse medicinal value (***Teh, C.H., et al., 2010***). *Eurycoma longifolia*, native to Burma, Indochina, Thailand, and Southeast Asia is a tall Simaroubaceous slender shrub-tree commonly found as an understroy in the lowland forests at up to 500 m above sealevel. *E. longifolia* known locally as 'Tongkat Ali' in Malaysia, 'Pasakbumi' in Indonesia, 'Cay ba binh' in Vietnam and 'Ian-don' in Thailand is popularly sought after in herbal remedies and has been frequently prescribed either as a single ingredient or as a mixture with other herbs. The roots of this plant are used as folk medicine for the treatment of aches, persistent fever, tertian malaria, sexual insufficiency, dysentery, glandular swelling, and as health supplements (***Kuo, P.C., et al., 2003***). In Malaysia, the roots of *E. longifolia* are widely prepared as additives in health supplements and beverages, e.g. isotonic drink, coffee and tea, to increase virility, libido and sexual prowess (***Teh, C.H., et al., 2010***). In some regions of Jawa (Indonesia), the plant is called 'Bidara pahit' and the roots are used for traditional treatment of dysentery and tertian malaria (***Mitsunaga, K., et al., 1994***). From the roots, several classes of compounds have been

identified and they included quassinoids, canthin-6-one alkaloids, -carboline alkaloids, tirucallane-type triterpenes, squalene derivatives, and biphenylneolignans. Some of these constituents were shown to possess cytotoxic, antimalarial, antiulcer, antipyretic, and plant growth inhibition activities *(Kuo, P.C., et al., 2003)*. In our continuing search for marker compounds from the root extract of *E. longifolia*, the chemical constituents of *E. longifolia* have been investigated. In this paper, we report the isolation 9-metoxyccanthin-6-one from methanol extract of the root of this species. This paper describes the isolation and structural elucidation of these compounds.

EXPERIMENTAL

a. Plant Material

Samples of the roots of *E. longifolia* were collected in April 2006.

b. General Experimental Procedures

^{1}H- and ^{13}C-NMR spectra were recorded with JEOL JNM ECA-500 spectrometer, operating at 500 MHz (^{1}H-) and 125.76 MHz (^{13}C-), using TMS (Tetra Methyl Silane) as an internal standard. MS were obtained with Mariner Biospectrometry spectrometer using System ESI (Electrospray Ionisation) and positive ion mode. Column chromatography was carried out using Merck Silica gel 60 (.70 - 230 mesh ASTM), and TLC (Thin Layer Chromatography) analysis on precoated Silica gel plates (Merck Kieselgel 60 F 254, 0.25 mm).

c. Extraction, Isolation and Identification

The dried roots (3.94 kg) of *E. longifolia* were extracted exhaustively using macerator with *n*-hexane. The residues from this extraction were macerated with methanol. Extracts methanol were concentrated using vacuum rotary evaporator and then fractionated by column chromatography on silica gel using gradient elution (hexane-ethyl acetate), resulted 9 fractions. Fraction 5 gave pure compound 1 as yellow crystal after re-crystallization.

RESULTS AND DISCUSSION

The dried roots of *E. longifolia* were macerated with *n*-hexane, and the residues from this extraction were macerated with methanol. The product of methanol extract was fractionated by column chromatography on silica gel using gradient elution (hexane-ethyl acetate) to give a number of fractions that contained a major compound as yellow crystal. This extraction had been resulted 9 fractions (***Figure 1***), which fraction 5 was re-crystallized to give pure ***compound 1***.

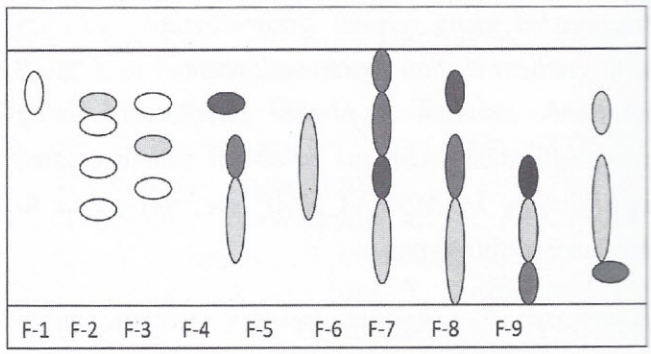

Figure 1. Thin Layer Chromatograpy Chromatogram from methanol extract of *E. longifolia* roots

Compound 1 was obtained as yellow crystal and determined as $C_{15}H_{10}N_2O_2$ by LC-MS spectrometry which $[M]^+$ ion at 250,2989 (***Figure 2***).

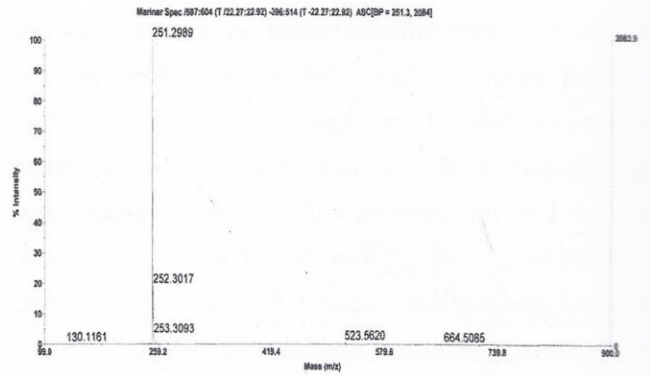

Figure 2. LC-MS Spectrum of compound 1 from methanol fraction of *E. longifolia* roots

The analysis of its NMR data, including HMQC and HMBC spectra, allowed for an unambiguous assignment of all proton and carbon signals (**Table 1 & Fig 3**). The ^{13}C-NMR spectrum indicated 15 carbons, including 7 sp^2 methine carbons, 1 methyl groups, and 7 quartenery carbons.

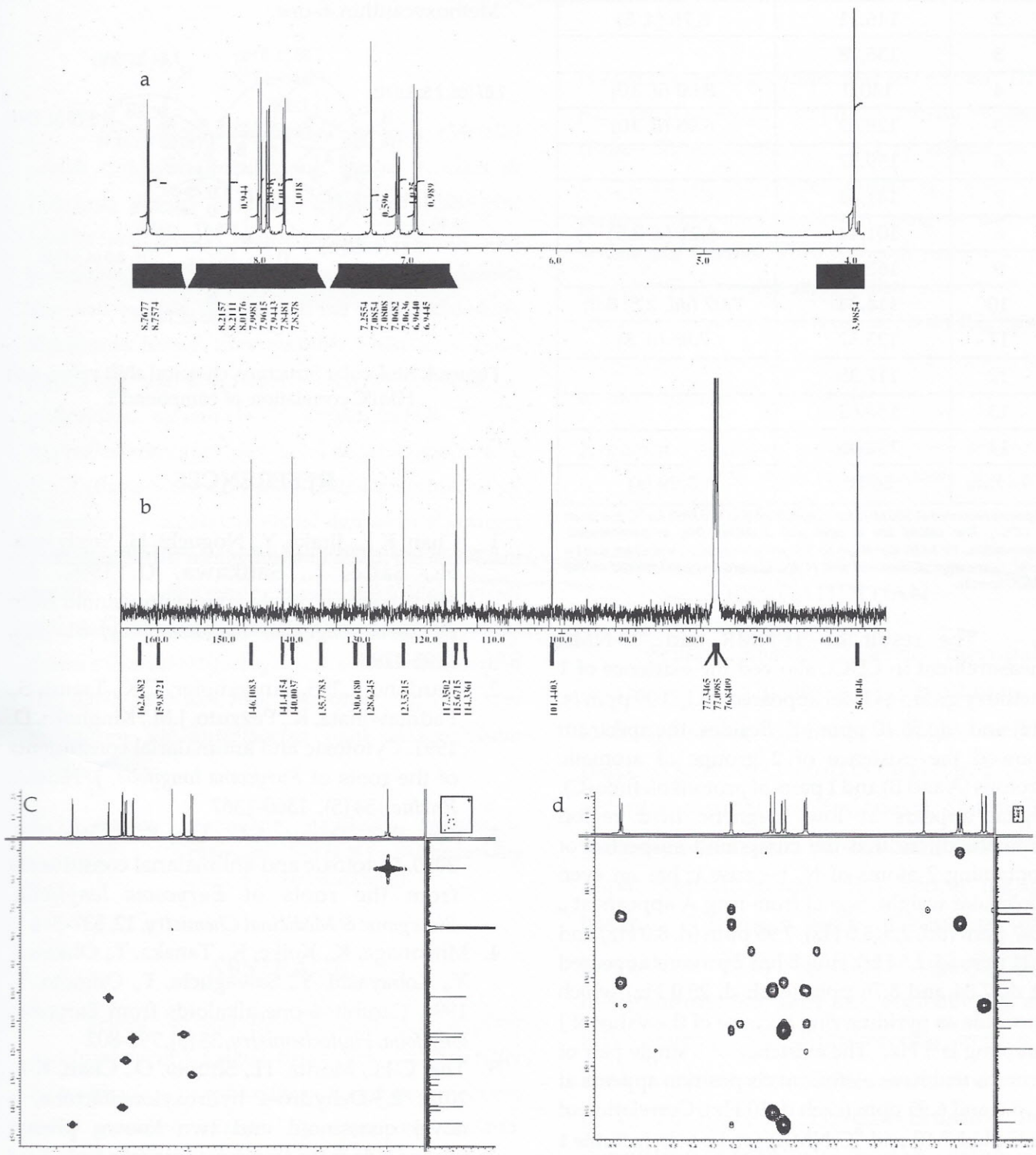

Figure 3. ^{1}H- (a), ^{13}C-NMR (b), HMQC (c), and HMBC (d) spectra from methanol extract of *E. longifolia* roots.

Table 1. ^{1}H- and ^{13}C assignment for compound 1 and correlation of H-C determined based on HMQC spectrum

No.	c	δ_H(*J* in Hz)
1	115,68	7,84 (*d*, 5)
2	146,11	8,76 (*d*, 5)
3	135,78	
4	140,0	8,00 (*d*, 10)
5	128,62	6,95 (*d*, 10)
6	159,57	
7	141,42	
8	101,44	8,21 (*d*, 2,5)
9	162,64	
10	114,34	7,07 (*dd*, 2,5; 8,0)
11	123,52	7,95 (*d*, 8)
12	117,35	
13	130,62	
14	132,45	
9-OMe	56,10	3,99 (s)

** Spectra recorded at 500 MHz for ^{1}H spectrum and 125 MHz for ^{13}C spectrum in $CDCl_3$. The values are in ppm and J values (Hz) in parentheses. Abbreviations for NMR signal are as follows: s = singlet, d = doublet, and t = triplet. Correlation of chemical shift H and C were assigned, based on the HMQC spectra.*

The result of ^{1}H-NMR and ^{13}C-NMR measurement in $CDCl_3$ showed the existence of 1 methoxy group (-OMe) appeared at d_H 3.99 ppm (s, 3H) and dc 56.10 ppm (q). Besides, the spectrum showed the existence of 2 groups of aromatic protons (A and B) and 1 pairs of protons olefinic (C). Signal appears at low magnetic field region (downfield), so that the compound suspected of containing 2 atoms of N, because it has an even molecular weight. Signal from ring A appears at $_H$ 7.07 ppm (dd, 2.5, 8.0 Hz), 7.95 ppm (d, 8.0 Hz) and 8.21 ppm (d, 2.5 Hz), ring B has 2 protons appeared at d_H 7.84 and 8.76 ppm (each d, 25.0 Hz) which conclude as pyridine ring because of the value of J coupling is 5 Hz. The existence of a single pair of protons that have olefinic at cis position appears at d_H 8.0 and 6.95 ppm (each d, 10 Hz). Correlation of H-C (^{1}H-NMR and ^{13}C-NMR) can be seen in **Table 1** based on the measurement of 2D-NMR spectrum (HMQC). Position-OMe group is located in ring A, based on measurement results HMBC correlation distance. Presence of carbonyl groups that are part of the D ring supported HMBC data as shown in **Figure 3.** Based on the above measurement results and literature data (Kardono, et al., 1991), the chemical content of *E. longifolia* is compound 1, is 9-Methoxycanthin-6-one.

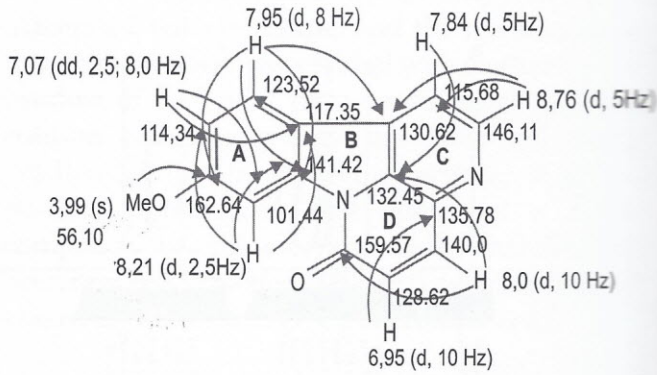

Figure 4. Molecular Structure, chemical shift value, and HMBC correlation of compound 1

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